PYRUVATE DEHYDROGENASE AND THE REGULATION OF GLUCOSE METABOLISM IN RUMINANT TISSUES

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1. Introduction

Ruminants absorb little glucose from their diet, >90% being synthesized by gluconeogenesis [1–3]. Glucose 'sparing' mechanisms are thought to operate in the ruminant to conserve glucose for essential purposes, since glucose oxidation contributes only 5–10% of CO_2 production [4] and the rate of fatty acid synthesis from glucose is very low in ruminant tissues [2].

Pyruvate dehydrogenase (PDH) is critically placed to regulate the irreversible loss of carbon from the pool of glucose and glucogenic precursors and its key role in this respect is well established in the rat [5]. The role of PDH in the control of glucose utilization in the ruminant has not been explored; the activity of the enzyme has not even been reported for most ruminant tissues. In this study we report the activity of PDH and the proportion in the active state (PDHa) for sheep adipose tissue, liver and placenta. The results suggest that PDH has an important role in the control of glucose utilization in sheep.

2. Materials and methods

Sheep were 10-month-old Finn X Dorset Horn crosses or pregnant 6–7-year-old Cheviot ewes: they were fed hay ad libitum plus a cereal mix (1 kg . day^{-1}). Sheep were killed with a captive-bolt humane killer. Samples of liver, perirenal adipose tissue or placenta were removed as quickly as possible and frozen in liquid nitrogen. Preparation of tissue extracts and assay of 'initial' (PDHa) and 'total' PDH activity were carried out as described by Stansbie et al. [6] except that the assays were performed at 37°C instead of 30°C. Protein concentration of the homogenates was determined by the method in [7]. The rate of incorporation of ^{14}C from [U-^{14}C]glucose into fatty acids in sheep adipose tissue slices was measured as in [8] except that the incubation medium (Krebs-Ringer bicarbonate) contained 3 mM glucose, 0.6 mM acetate, 1 mM L-lactate and 0.1 μg . ml^{-1} insulin.

3. Results and discussion

Preliminary studies were carried out using tissues from young (170–190 g) female Wistar rats to ensure that the PDH assay was satisfactory in our hands. For liver, total PDH activities of 1.30 and 1.34 μmol . min^{-1} . g $tissue^{-1}$ were found which, allowing for differences in assay temperature, are very similar to those reported for rat liver [6,9,10]; in addition 16–17% of the activity was in the active state, again in good agreement with [9,10].

3.1. *Adipose tissue*

The total PDH activity of perirenal adipose tissue from young sheep (table 1) was <20% of that of epididymal adipose tissue of young rats (200–400 nmol . min^{-1} . g^{-1} or 16–20 nmol . min^{-1} . mg $protein^{-1}$, at 30°C) [6]. In addition only 19% of the PDH in sheep adipose tissue was in the active state compared with 40–50% in adipose tissue from rats fed normal chow [8], but this is similar to the proportion of PDH in the active state in fasted or diabetic rats or rats fed a high fat diet for 6 days (12–18%) [6]. Adipose tissue from rats in these latter states has a low rate of fatty acid synthesis from glucose and a relatively high proportion of glucose utilized is released from the tissue as lactate [6,11,12].

Table 1
Pyruvate dehydrogenase activity of sheep tissues

Animal	Tissue	No. obs.	Pyruvate dehydrogenase activity				Initial / Total × 100
			nmol . min^{-1} . g tissue^{-1}		nmol . min^{-1} . mg protein^{-1}		
			Initial[a]	Total	Initial	Total	
10-month-old	Adipose tissue	5	8.9 ± 3.3[b]	45.1 ± 9.4	1.4 ± 0.8	6.4 ± 2.5	19 ± 2.4
	Liver	5	162.2 ± 45.3	339.1 ± 43.1	0.8 ± 0.3	1.5 ± 0.3	48.2 ± 7.57
Pregnant (−30 days pre-partum)	Placenta	3	293.7 ± 25.6	392 ± 117	2.3 ± 0.8	4.6 ± 1.3	82.6 ± 3.7
Pregnant (−2 days pre-partum)	Placenta	9	141 ± 30	172.7 ± 41.6	2.0 ± 0.6	2.5 ± 0.8	66.4 ± 8.6

[a] Initial ≡ PDHa
[b] Values expressed as mean ± S.E.M.

The activity of PDHa (table 1) was the same as that of ATP-citrate lyase of sheep adipose tissue (5–9 nmol . min^{-1} . g tissue^{-1}) [13,14]. This latter enzyme has long been considered to be the primary reason for the low rate of fatty acid synthesis from glucose in ruminant tissues [2,13]; the present results suggest that PDH also has an important role in this respect. This view is supported by the observation that ~50% of the glucose metabolized by ruminant adipose tissue both in vivo and in vitro is released as lactate [15–17]. In addition the rate of fatty acid synthesis from glucose was measured in adipose tissue slices from the sheep (the rate ranged from 1–9 nmol glucose converted . h^{-1} . g tissue^{-1}). Regression analysis showed a highly significant correlation between the rate of fatty acid synthesis from glucose and the activity of PDHa ($r = 0.991, P < 0.001$). These various observations indicate that glucose metabolism in sheep adipose tissue is most probably restricted by a low PDHa activity and this may be as important as the low ATP-citrate lyase activity in limiting the flux of glucose carbon to fatty acids. This is probably true for ruminant mammary gland also, for a relatively low 'total' PDH activity has been reported for this tissue [18].

3.2. *Liver*

The total PDH activity of sheep liver (table 1) was ~25% of that of rat liver [9,10], however, unlike the rat liver enzyme (see above) 48% of the PDH was in the active state, hence the activity of PDHa is similar in sheep and rat liver. The reason for the high proportion of PDH in the active state in sheep liver is not known. As gluconeogenesis is the dominant pathway of glucose metabolism in ruminant liver [1–3] PDH is unlikely to be required for glucose oxidation. It may, however, provide a mechanism for the net oxidation of propionate (and some amino acids) to CO_2 as this requires prior conversion to acetyl CoA. Propionate, albeit at 5 mM, increased the activity of PDH in rat adipose tissue [19]. Propionate in the hepatic portal vein of sheep is ~0.2 mM and most of it is taken up by the liver [20].

3.3. *Placenta*

During late pregnancy glucose uptake by the uterus and placenta accounts for ~50% of the glucose turnover rate [21] but only 30% of this is used by the foetus, the rest being utilized by the uterus and the placenta [22]. Of the glucose, 15% can be accounted for as CO_2 and 37% as lactate released into the circulation [22]. This suggests a low PDH activity in the placenta. The PDH activity of rat placenta does not appear to have been reported, but total PDH activity per gram tissue of sheep placental cotyledons is <25% of that of a variety of rat tissues (with the exception of adipose tissue) [6,19]. The activity fell by 50% towards term but the proportion in the active state,

which was relatively high, did not change. By late-pregnancy the weight of the empty uterus plus placenta is ~1.2–1.3 kg in sheep with a single lamb [23] while the weight of the placental cotyledons is ~0.4 kg [24]. Unless the PDHa activity of the other constituents of the utero–placental unit is markedly greater than that of the cotyledons, the PDHa activity during late-pregnancy appears to be sufficient to metabolize only 50% of the glucose utilized at this time (200 μmol . min^{-1}) [22].

4. Conclusions

The results of the present study along with those of Stansbie et al. [6] and Read et al. [18] show that the total PDH activity of ruminant tissues is <25% of that of the rat which is in accordance with the low rate of glucose oxidation to CO_2 in ruminants. Total PDH activity and, in the case of adipose tissue at least, the proportion in the active state, are especially low in ruminant adipose tissue and mammary gland, tissues with a high lipogenic capacity [25] and this is probably a major cause of the low rate of fatty acid synthesis from glucose.

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